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Gypsy Moth Fungus

Entomophaga maimaiga in North America: A Review

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Introduction

The gypsy moth, *Lymantria dispar* (L.), is a serious defoliator of broadleaved forests in eastern North America. Populations of this insect pest undergo periodic outbreaks increasing to high densities that result in widespread defoliation to an average 2.0 million forested hectares (ha) per year. The gypsy moth is endemic throughout temperate Eurasia and was introduced into the Boston area of Massachusetts in 1869. This pest has spread to the southwest and continues to spread at the rate of 6 to 9 km per year.

In eastern North America, the gypsy moth is subject to a variety of naturally occurring infectious diseases caused by several kinds of bacteria, fungi, and a nucleopolyhedrosis virus (NPV), which was inadvertently introduced with gypsy moth or its parasites (Campbell and Podgwaite 1971). There are six endemic species of entomopathogenic (causing disease in insects) fungi known to infect the gypsy moth (Majchrowicz and Yendol 1973, Hajek et al. 1993).

The fungal class Zygomycetes, which includes the bread molds, is a primitive group of fungi with no species native to North America known to infect gypsy moth. Species in one zygomycete order, the Entomophthorales, predominantly specialize as insect pathogens. Many entomophthoralean pathogens are known to cause dramatic epizootics (disease outbreaks) in insect populations. *Entomophaga* (separated from the older genus *Entomophthora* in 1984 by Humber) *aulicae* is a complex of species, all of which attack Lepidoptera (moths and butterflies). North American strains of the *E. aulicae* complex are known to infect species in nine families within the Lepidoptera, including Noctuidae (e.g., cutworms), Arctiidae (e.g., fall webworm), and Lymantriidae (the tussock moths) (Hajek et al. 1991b). Yet, native *E. aulicae* species have never been reported from North American popula-

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tions of two important lymantriids, the gypsy moth and Douglas-fir tussock moth, *Orgyia pseudotsugata*. Strains within the *E. aulicae* complex can be specific to certain Lepidoptera, but since all members of this complex are morphologically identical, strains must be differentiated using biochemical tests.

In Japan, epizootics of an entomophthoralean fungus have frequently been reported from high density populations of gypsy moth (Koyama 1954, Takamura and Sato 1973). In 1908, pest managers attempting to control expanding gypsy moth populations in the Northeast first heard of this effective Japanese fungus. Gypsy moth larvae infected with this entomophthoralean fungus were collected from Nishigahara, near Tokyo, Japan, in 1909 and brought to the United States for evaluation as a gypsy moth control. This fungus appeared to be a member of the *E. aulicae* species complex. In 1910-1911, larvae infected with the "gypsy fungus" were released at six sites near Boston (Speare and Colley 1912). No transmission of this disease resulted due, in part, to unfavorable weather conditions and the occurrence of an NPV outbreak. When the local gypsy moth population collapsed in 1911, there was no way to continue propagating the fungus (the gypsy moth could not be reared in the laboratory over the winter and the maintenance of fungal cultures failed due to reliance on overwintering resting spores produced by late instar larvae). Therefore, this project was discontinued.

In 1984, Soper and Shimazu isolated this entomophthoralean fungus from the Japanese gypsy moth and brought isolates to the United States. Stages of this fungus now could be maintained year round in the laboratory using several different culture media, rather than having to be perpetuated on the host. The morphological characteristics of the Japanese isolates were identical to *E. aulicae* strains, yet only the Japanese fungus could infect gypsy moth. Since the isozyme pattern, distribution, and host range of this fungus differed from those of other isolates within the *E. aulicae* species complex, the name *Entomophaga maimaiga* was given to the Japanese isolates (Soper et al. 1988). The specific name for this new species, "maimaiga," was based on the Japanese common name for the gypsy moth. Additional analyses confirmed differentiation of *E. maimaiga* within the *E. aulicae* species complex (Hajek et al. 1990b).

Isolates of *E. maimaiga* from Japan were evaluated in the laboratory and one isolate was selected for field release based on its ability to cause a high percentage of mortality in gypsy moth larvae between 15 and 25°C, and the shorter time from infection to death of hosts, compared with other isolates (Shimazu and Soper 1986). This Japanese isolate was released as larvae injected with fungal protoplasts in 1985 in Allegany State Park, New York, and in 1986 in Shenandoah National Park, Virginia to experimentally study disease transmission. Researchers hoped that infected larvae would die, produce spores, and transmit *E. maimaiga* to the wild populations of gypsy moth. In 1985, there was only limited transmission (0.08%) of *E. maimaiga* from infected larvae to the total of 3698 native gypsy moth larvae sampled in the year of release. In 1986, no infection was detected at Allegany State Park. The low level of transmission by *E. maimaiga* probably occurred because NPV was prevalent in both the field-collected larvae injected with protoplasts to produce the disease inoculum and the larvae sampled to detect resulting infection. For the 1986 release in Shenandoah National Park, *E. maimaiga*-infected larvae originated from a laboratory colony reared on artificial diet. A total of only 0.38% of the 3409 wild gypsy moth larvae collected in 1986 were infected, possibly due to minimal rainfall hampering disease transmission. No *E. maimaiga* infections were detected at fungal release sites in 1987 and 1991 in Shenandoah National Park, or in 1989 and 1990 in either Park.

Biology

Resting spores (azygospores) (Fig. 1) are the overwintering life stage of *E. maimaiga* and have an obligate dormant period. Beginning in spring, upon favorable conditions for germination, resting spores begin producing infective, pear-shaped germ conidia (Hajek and Roberts 1991). Germ conidia are actively discharged, and they or their progeny land on the skin of larvae. Enzymes assist the fungus in penetrating into the caterpillar's body. The fungus reproduces as a protoplast stage (Fig. 2) in the hemolymph, by using nutrients in the blood. Shortly before infected larvae die, the fungus invades the vital organs. Under constant temperatures from 20-25°C, it takes less than one week for a caterpillar to die (Shimazu and Soper 1986), with feeding decreasing a few days prior to death (Hajek 1989). After larvae die, hyphal bodies form in the hemolymph, leading to production of either conidiophores,

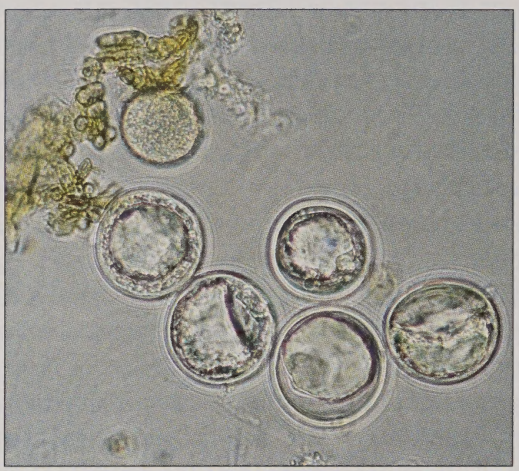

Figure 1. Overwintering resting spores (azygospores) of *Entomophaga maimaiga*.

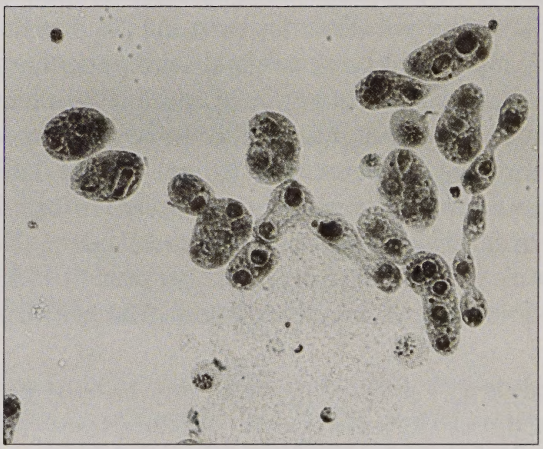

Figure 2. Protoplasts of *Entomophaga maimaiga* that occur within the hemolymph of infected insects.

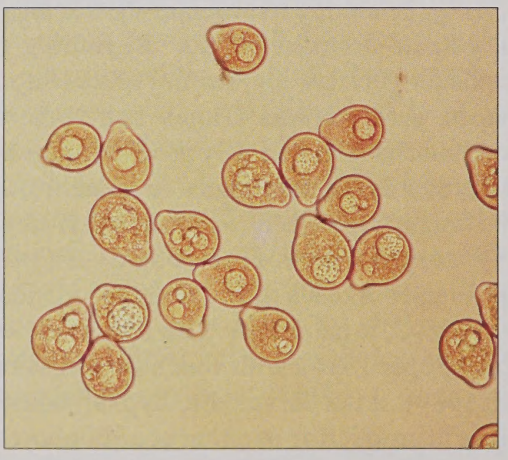

Figure 3. Conidia of *Entomophaga maimaiga* actively ejected from cadavers to cause infection during the same season.

that grow out through the integument and actively release pear-shaped conidia (Fig. 3), or of internal resting spores, or of both.

Laboratory results demonstrate that early instars of gypsy moth predominantly produce conidia, the spore type that induces disease spread within a season, while later instars usually produce resting spores and sometimes conidia also. Neither type of spore can be seen with the naked eye. In the laboratory, third and fourth instar larvae are most susceptible to the fungus, although high percentages of all instars become infected. The relatively short-lived conidia are actively ejected from cadavers and infect hosts as do germ conidia. Cadavers of late instars frequently remain attached to tree boles, and resting spores within cadavers are gradually leached onto the tree bark or into the soil, where they remain in a dormant state throughout the fall and winter. Resting spores germinate throughout springtime, but maximum germination usually occurs one to two days after significant precipitation. In contrast, the greatest production of conidia from cadavers of larvae killed by *E. maimaiga* takes place on the day of rainfall (Weseloh and Andreadis 1992). Cadavers also discharge conidia in response to dew or extended relative humidities of > 70% (Hajek and Soper 1992).

Two crucial stages in the transmission of fungal pathogens of insects are the production of conidia from moribund hosts and subsequent changes in conidia from disseminating propagules to infectious agents (i.e., germination). In laboratory studies, high humidities are necessary for conidial production and discharge and free water is necessary for conidial germination (Hajek et al 1990a). Moisture levels therefore act as a switch making germination possible or not. The optimal temperature for *E. maimaiga* germination, initiation of sporulation and disease incubation is approximately 20°C. Therefore, under optimal moisture conditions *E. maimaiga* can undergo from 4-9 multiplicative cycles within one generation of the gypsy moth host depending on spring temperatures between 14-26°C.

Disease Symptomatology

E. maimaiga and NPV are the principal natural enemies of gypsy moth that kill large numbers of larvae. Cadavers of larvae killed by both

diseases remain hanging on tree boles. Cadavers of late instar larvae killed by *E. maimaiga* are often oriented vertically with heads down, all prolegs frequently at a 90° angle to the axis of the body, and bodies tightly attached to tree boles (Fig. 4). Larvae recently killed by the fungus are soft-bodied, and older cadavers appear dry (Hajek and Roberts 1992). By contrast, larvae killed by NPV are predominantly positioned with anterior prolegs attached to the bole, the anterior section of the body hanging unattached, and the body bending at an angle of less than 90° (Fig. 5).

Figure 4. Cadaver of a late instar gypsy moth filled with *Entomophaga maimaiga* resting spores. Note the remains of some of the conidia attached to larval hairs, the dried appearance of the cadaver, and the vertical position with head down. *Photo by D. Specker.*

Figure 5. Cadaver of a late instar gypsy moth killed by NPV. Note the moist appearance of this older cadaver and the inverted "V" position. *Photo by D. Specker.*

Cadavers of NPV-killed larvae usually remain soft and moist, and the integument is easily ruptured. The body contents of cadavers recently killed by *E. maimaiga* are liquefied and usually filled with a mixture of hyphal bodies, immature resting spores and a few mature resting spores (Fig. 6A). Cadavers producing conidia are covered with a white to brown velvet-like mat of conidiophores. After conidial production, the fungal growth on the cadaver surface decomposes, but remains of conidia can sometimes be found attached to hairs (Fig. 6B). Hajek and Roberts (1992) found about 4% of the analyzed cadavers with mixed infections of both fungus and NPV. These external characteristics described above are not sufficiently reliable for diagnosis; therefore, larvae should be dissected. Most fungal-killed cadavers fall from tree boles within 9-10 days after death (Hajek and Soper 1992), but some remain attached to trees all winter (Fig. 6C).

Infection by *E. maimaiga* can be confirmed by using enzyme-linked immunosorbent assay (ELISA) of larvae (Hajek et al. 1991a), by promoting sporulation from cadavers in humid chambers, or by dissecting cadavers. To observe cadaver contents, soak cadaver in water, remove a small piece, and place it in a drop of water on a microscope slide. Cover with a cover slip. Dissected material is easily observed at 100-400 magnification on a compound microscope. Conidia are pear-shaped and average 20 x 25 micrometers. Resting spores average 30 micrometers in diameter and have a thick double wall.

Population Dynamics

Epizootics of the gypsy moth fungus are initiated in the spring when early stage larvae are infected by resting spores found in leaf litter, bark and soil. Infected larvae die and, if moisture conditions are adequate, discharge conidia, which infect healthy larvae. Since *E. maimaiga* does not occur in all areas where gypsy moth occurs, techniques for introducing *E. maimaiga* were evaluated using different fungal life stages (conidia and resting spores) and manipulating the habitat into which the spores were introduced.

In 1990, seven plots (each 0.01 ha) with oak as the dominant species were established in Ithaca, NY (Hajek and Roberts 1991). *E. maimaiga* was introduced using four methods: (1) field-collected soil containing

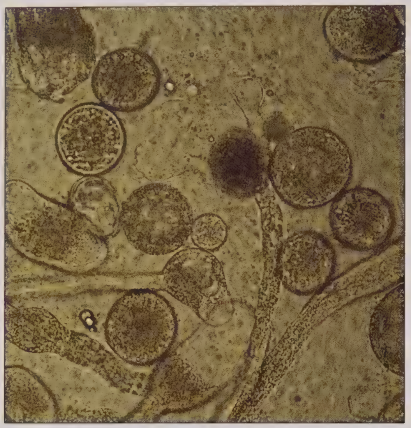

Figure 6A. Fungal hyphal bodies and immature resting spores within a recently killed gypsy moth larva.

Figure 6B. Cadaver of a gypsy moth larva producing abundant conidia, some of which remained attached to larval hairs.
Photo by D. Specker.

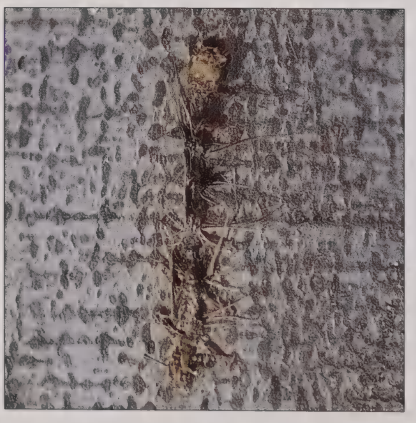

Figure 6C. Cadaver of a gypsy moth larva killed by *Entomaphaga maimaiga* and still attached to a tree trunk in spring.

resting spores placed around the base of a tree and not allowed to dry for more than 1 day (sprinkled with water); (2) same as (1) although receiving only ambient moisture; (3) same soil placed in a band at a height of 2m on tree boles and not allowed to dry for more than 1 day; and (4) infected larvae released on the bole of the central tree. Control plots were untreated. The first two treatments were planned to induce infections by resting spores as the mobile gypsy moth larvae moved within and among trees. The third treatment was planned to expose late stage larvae to resting spores during the day when they accumulate in cryptic locations. The fourth treatment was designed to produce conidia on cadavers resulting from the infected larvae that had been released. Conidia actively ejected from cadavers would then potentially disperse through the tree canopies as well as infect healthy larvae resting near cadavers.

Northeastern strains of *E. maimaiga* were used for these studies. For the first three treatments, soil containing resting spores was collected in southeastern New York State. In May, 1.18 kg of soil containing approximately 1.18×10^6 resting spores was distributed around bases of individual trees in treatments (1) and (2), shortly after gypsy moth eggs began to hatch. Soil containing a total of 8.9×10^5 resting spores was enclosed in organdy bags under burlap bands on tree boles in treatment (3). For treatment (4) larvae from a laboratory colony were injected with *E. maimaiga* to evaluate the occurrence of disease caused by only the short-lived conidial stage. A total of 150 fungal protoplasts of a northeastern isolate of *E. maimaiga* was injected into each of 500 fourth instar larvae, which were released on tree boles 1 day after injection at two sites, on June 5.

Infection levels from treatment (1) surpassed those from all other introduction methods. High infection levels were associated with subsequent lower gypsy moth densities, although in most plots the gypsy moth populations still increased in1989 and 1990.

In this study, for treatments(1) to (3) in which resting spores were released, *E. maimaiga* could have caused infections in two ways: (1) from the production of infective germ conidia by resting spores (primary infection), and (2) from the production of conidia on cadavers of

gypsy moth larvae that had themselves become infected (secondary infection). Little infection was seen in early instars, whereas infection levels reached higher proportions in the later instars.

Distribution (1989-1993)

E. maimaiga does not occur in all areas where the gypsy moth occurs, especially in those areas more recently colonized by the gypsy moth (Hajek et al. 1990b). This fungus is prevalent in low-to-high density gypsy moth populations, causing up to 100% mortality of late stage larvae. It is highly variable, and as yet unpredictable, in reducing gypsy moth populations.

In June and July 1989, *E. maimaiga* was first recovered in North American gypsy moth and was found causing extensive epizootics in increasing populations of gypsy moth in seven contiguous northeastern States (Connecticut, Massachusetts, Vermont, New Hampshire, New Jersey, New York, and Pennsylvania) (Andreadis and Weseloh 1990, Hajek et al. 1990b) (Fig. 7). By 1990, *E. maimaiga* also was recovered in three more northeastern States (Maine, Delaware, Maryland) and in southern Ontario (Elkinton et al. 1991, Welton 1991). *E. maimaiga* was not recovered from larvae collected from Virginia, West Virginia, western Maryland and western Pennsylvania, despite the fact that these regions had equally rainy conditions. The prevalence of *E. maimaiga* in 1989 and 1990 was probably due in part to above-average precipitation in May, coincident with increasing gypsy moth populations. Despite below-average precipitation in May and June 1991, however, *E. maimaiga* was recovered at numerous sites and caused some epizootics. Based on high prevalence and widespread distribution far from fungal release sites, these epizootics during 1989-1991 apparently were not the result of 1985 and 1986 introductions. The results of the 1990 study evaluating methods for introducing the northeastern United States strain of *E. maimaiga* were critical for efforts to introduce the fungus using overwintering resting spores on a larger scale.

The Appalachian Integrated Pest Management Gypsy Moth Project (AIPM Project), in cooperation with the Forest Service's Northeastern Forest Experiment Station, provided funding for release of *E. maimaiga* both within the AIPM Project area (Hajek 1991-1993) and outside the

project area (Elkinton 1991-1993). In 1991, *E. maimaiga* resting spores collected in central Massachusetts were released in 0.01-ha plots in Pennsylvania, West Virginia, Virginia and Maryland along the leading-edge of gypsy moth infestations. Infections were recovered in 28 of the 34 release sites with epizootics at some sites. In 1992, *E. maimaiga* resting spores collected in central New York were released in seven plots in Virginia and West Virginia. High levels of infection were found at nearly all release sites for both 1991 and 1992. The objectives of releasing *E. maimaiga* in 1991 and 1992 were to investigate fungal establishment and impact, fungal spatial distribution, dispersal, behavior of infected larvae, and interaction with other biotic components; control was not a study objective.

Epizootics of *E. maimaiga* occurred in 1992 in New England gypsy moth populations, as well as in most of the 1991 and 1992 newly established release sites in Virginia and West Virginia. The distribution of this fungus continues to expand in areas more recently colonized by gypsy moth. Introduction efforts along the southern and western edges of gypsy moth distribution have obscured the rate of natural spread of *E. maimaiga,* although spread of greater than 1-km was recorded between consecutive years at numerous release sites. This fungus is now so widespread in Virginia that it is difficult to determine whether the fungus at an individual location is the result of spred from a release or of natural migration from the north where it is established (Fig. 8). Limited introduction of *E. maimaiga* in Michigan gypsy moth populations demonstrates similiar trends of establishment and spread (L. Bauer and D. Smitley, pers. comm., 1993).

When *E. maimaiga* was first found in North America in 1989, the simplest explanation was that the 1910 and 1911 introductions had been successful in establishing the fungus, which later produced local infections resulting in its subsequent spread. *E. maimaiga* may not have been detected between 1911 and 1989 because cadavers of larvae killed by *E. maimaiga* look similar to cadavers of larvae killed by NPV to untrained observers. Conidia and conidiophores present on some cadavers are very short-lived, and usually only microscopic examination can confirm presence of *E. maimaiga.* The rapid spread of *E. maimaiga* documented from 1989 to 1992 poses questions regarding the

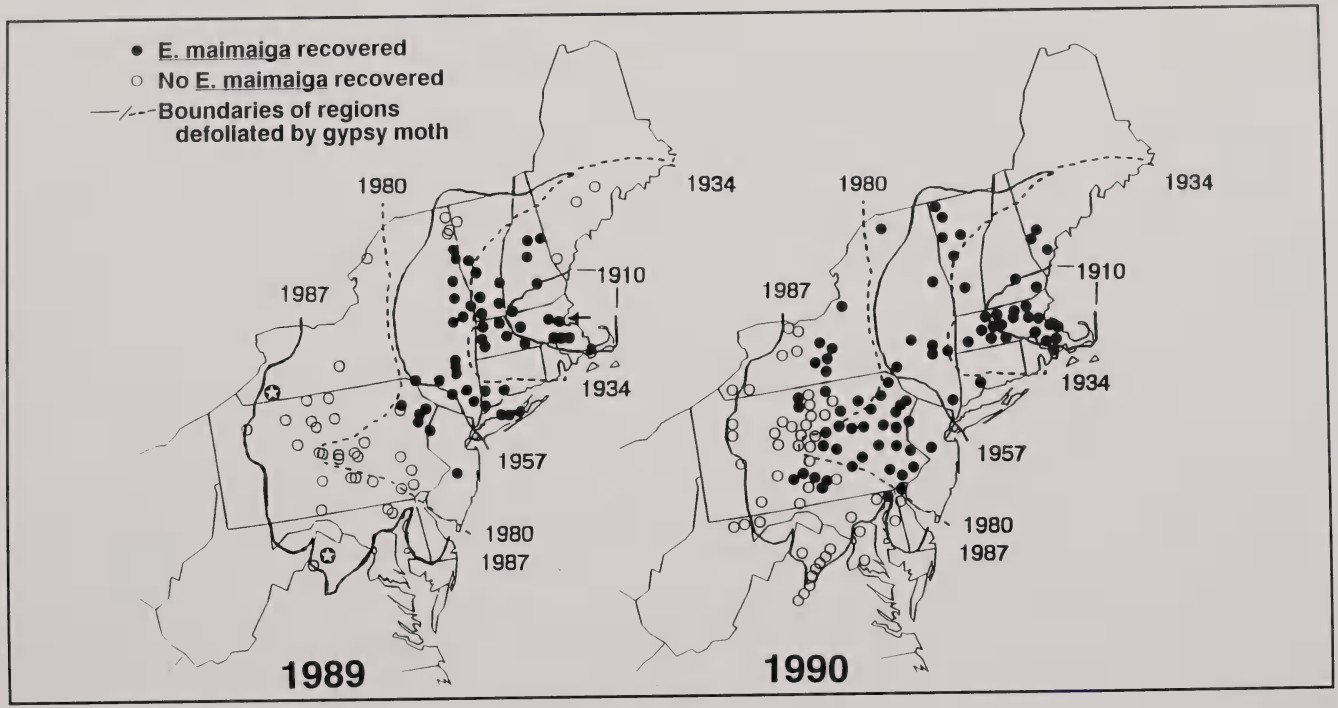

Figure 7. Distribution of *Entomophaga maimaiga* in the United States during 1989 and 1990. Dated concentric lines indicate the spread of gypsy moth defoliation. Stars indicate locations of 1985 and 1986 releases of the 1984 Japanese isolate of *E. maimaiga* (Elkinton et al. 1991).

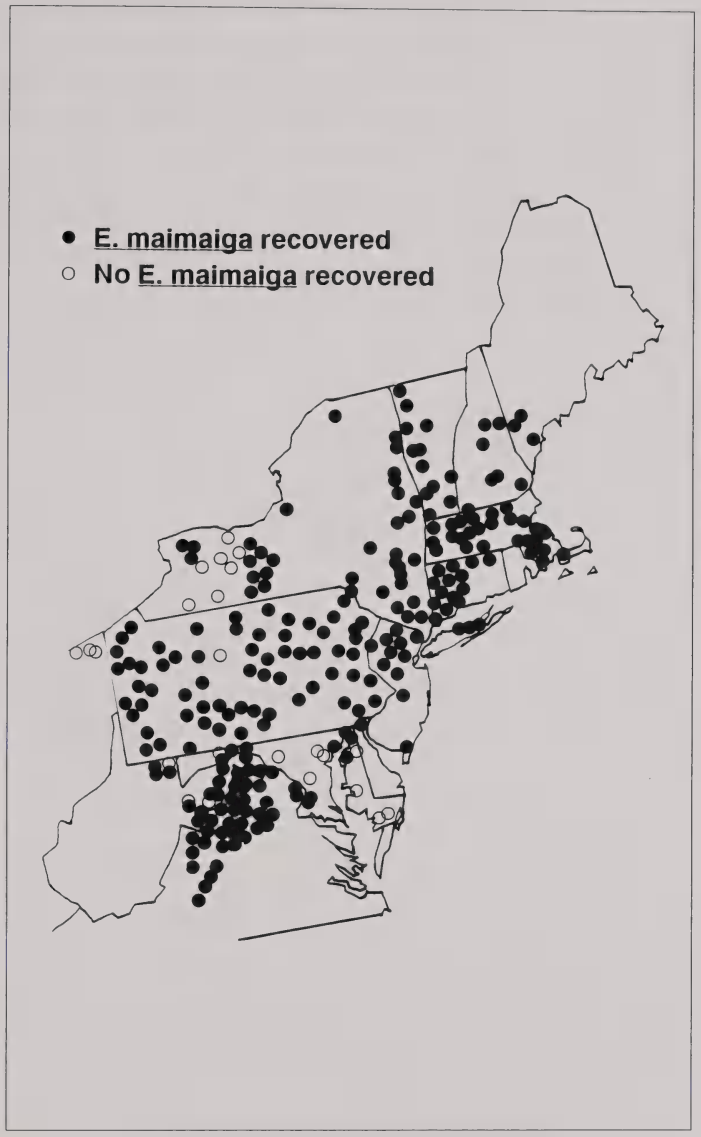

Figure 8. Distribution of *Entomophaga maimaiga* in the United States from 1989 to 1992, including sites where *E. maimaiga* was absent in 1992.

successful establishment of this fungus during releases in 1910 and 1911. If the fungus can spread as quickly as recorded from 1989-1992, it is difficult to imagine that it required 79 years to spread from Boston to its 1989 distribution. The strain of *E. maimaiga* introduced in 1910-1911 was considered weak, and the introduction project was considered a failure and discontinued. The strain of *E. maimaiga* introduced in 1910-1911 may have barely survived in the environment for many years, and then a more highly pathogenic strain evolved and began spreading. Another hypothesis would be that *E. maimaiga* did not establish at all in 1910-1911 but was more recently accidentally introduced to North America.

Use as a Mycoinsecticide

Spread — The relocation of *E. maimaiga* resting spores and its habitat soil from one location to another requires obtaining the necessary permits (e.g., form PPQ 526) from the USDA Animal and Plant Health Inspection Service (APHIS) and taking additional precautions to ensure that plant pathogens (e.g., *Armillaria mellea* rhizomorphs) and arthropod pests are not unintentionally spread. Therefore, laboratory production of *E. maimaiga* on artificial media would provide a method of producing quantities of *E. maimaiga* without the potential of introducing pest species in soil.

Laboratory production — Among the entomophthoralean fungi, resting spores have been suggested as an excellent life stage for use in pest control. Most attempts to induce epizootics by manipulating resting spores, however, have proven unsuccessful (Hajek and Roberts 1991). However, several systems for mass-producing resting spores in the laboratory have been developed for entomophthoralean pathogens of aphids (Soper et al. 1975, Latgé et al. 1977).

Conidia are not a suitable host stage for pest control use as they are fragile and short-lived under normal storage conditions. Since mycelia (multinucleate vegetative structures) are more easily produced, a system was developed with entomophthoralean fungi for producing dry, viable mycelia that are storable at refrigerator temperatures for long periods. Mycelia are produced in an aerated liquid medium, harvested by filtration, and dried with a sugar desiccation protectant. The ability

to grow the mycelial phase of *E. maimaiga* in liquid media would provide the potential for large-scale production and the use of a dried mycelial process for producing it as a mycoinsecticide (McCabe and Soper 1985). The dried mycelia would be milled into a powder, stored at low temperature, and introduced into the field, where, upon contact with free water (dew) they would rehydrate and produce conidia to infect gypsy moth larvae. Although only very limited trials have been conducted to date, attempts to grow *E. maimaiga* in liquid media have been unsuccessful (Soper et al. 1988). Mass fermentation methods have been developed, however, for other *Entomophaga aulicae* strains (Nolan 1993).

Constraints on Operational Use — Numerous constraints limit the development of entomopathogenic fungi for use as mycoinsecticides. Foliar applications of fungi are very sensitive to abiotic factors (humidity, degradation by ultraviolet light and solar heat, removal from target habitat by rainfall). Compared with insecticides, fungi can be relatively slow to kill the host (at least 1 week). Fungi are also often short-lived in storage and relatively expensive to produce. Host specificity of many entomophthoralean fungi make their production for control more expensive although making them more desirable due to decreased nontarget impact. Dried-mycelium preparations will present unique formulation problems of adhering the larger particles to leaf surfaces while protecting them from adverse environmental conditions.

Host Range

There is extensive public interest in the host specificity of *Entomophaga maimaiga* both because it is spreading rapidly and because it may be developed for gypsy moth control in the future. Host range studies have shown that *E. maimaiga* does not infect insects other than Lepidoptera. *E. maimaiga* was specifically tested on caged adult honey bees and did not affect longevity or cause disease (Vandenberg 1990). Within the Lepidoptera, *E. maimaiga* is a virulent pathogen of both the gypsy moth and Douglas-fir tussock moth, whether hosts are exposed to conidia or injected with protoplasts (Soper et al. 1988). The specificity of *E. maimaiga* for *L. dispar* has been demonstrated in transmission tests (Soper et al. 1988). Native gypsy moth larvae showed susceptibility only to isolates of *E. maimaiga* originating from Japanese gypsy moths

and never were infected successfully by any North American isolates of *Entomophaga* from other lepidopteran hosts.

Non-targets -The AIPM Project funded laboratory evaluations of potential effects of *E. maimaiga* on nontarget Lepidoptera. Larvae of 43 species were collected in West Virginia; five suborders were represented. Laboratory bioassays were conducted after both conidial inoculation (larvae were dipped into a conidial suspension) and injection of protoplasts. Of the 5 suborders tested, *E. maimaiga* infected three (Noctuoidea, Bombycoidea, and Geometroidea). For all families except Lymantriidae (suborder Noctuoidea), low infection levels occurred in only some of the species tested (Table 1) (Hajek and Butler, unpublished data).

Gypsy moth larvae are present only for approximately 9-10 weeks each spring or early summer, and many lepidopteran larvae are not active during this time. *E. maimaiga* resting spores germinated from approximately 2 weeks before gypsy moth eggs began to hatch through 2 weeks after most gypsy moth larvae had pupated. Therefore, only lepidopteran species with larvae active around the time that gypsy moth larvae are active potentially could become infected.

Research and Methods Development

Additional research is critically needed in the areas of field ecology, biology and population dynamics of *E. maimaiga,* before mycoinsecticide development and field application. There are many unanswered questions concerning *E. maimaiga.* What factors trigger germination of resting spores in various microhabitats, influence larval infection and disease incubation period, and affect spatial and temporal patterns of spore dispersal? How are disease transmission and spread influenced by host and pathogen densities and biological interactions between this fungus and NPV? The effects of *E. maimaiga* on nontarget organisms are being evaluated further; these data are a first-order requirement for development of any control agent.

Identification of fungal strains for commercial laboratory production will require data on fungal survival in various habitats, growth and

sporulation characteristics, and genetic stability, as well as pathogenicity and virulence. A commercially acceptable method of laboratory production must be developed as well as an appropriate formulation. A standard quantitative bioassay procedure, similar to standardized *Bacillus thuringiensis* bioassays, must be developed to optimize any potential *E. maimaiga* product to be used for control.

Studies with *E. maimaiga* have been conducted for a relatively brief period and have only just begun to identify the information on host-pathogen interactions that is vital to developing this fungus for effective biological control.

Summary

In 1909, gypsy moth larvae infected with a fungus were collected from Japan and brought to North America. In 1910-1911, larvae infected with this "gypsy fungus" were released near Boston, Massachusetts. No fungal infections resulted. In 1984, Soper and Shimazu isolated a fungus, *Entomophaga maimaiga*, from the Japanese gypsy moth. This Japanese isolate was released in 1985 in Allegany State Park, New York, and in 1986 in Shenandoah National Park, Virginia. *E. maimaiga* killed cadavers were not detected in 1989 and 1990 at both parks, and in 1987 and 1991 at Shenandoah National Park release sites.

In June and July 1989, *E. maimaiga* was first recovered in North American gypsy moth and found causing extensive epizootics in northeastern States. By 1990, this fungus was recovered in 10 northeastern States and in southern Ontario. *E. maimaiga* did not occur in areas more recently colonized by the gypsy moth, and the Japanese strain was not recovered after the 1985 and 1986 releases. Therefore, the northeastern United States strain was released in 1991 and 1992 along the leading-edge of gypsy moth spread in West Virginia, Virginia, Maryland and western Pennsylvania. *E. maimaiga* established readily at release sites and spread rapidly.This fungus is now so widespread in the Northeast that it is difficult to determine at individual locations whether the fungus originated from a release site or from natural migration.

The need to conduct additional research is critical in the areas of field ecology, biology, epizootiology (study of disease dynamics) and natural population dynamics of *E. maimaiga* before the development and

application of this fungus as a mycoinsecticide. The present method of introducing resting spores of the fungus along with its habitat soil is viable only for small-scale, highly regulated use with careful attention to associated concerns of spreading plant pathogens and arthropod pests.

The use of *E. maimaiga* as a mycoinsecticide will require the development of commercial cost-effective methods to grow the resting spores or mycelial phase in media. Also, a formulation must be developed that will protect the fungus from abiotic factors (e.g., degradation by ultraviolet light).

E. maimaiga is effective in both high- and low-density gypsy moth populations, unlike the nucleopolyhedrosis virus, which is only effective at high-density populations. The fungus could play a significant role in the natural control of gypsy moth, especially in years with a wet spring. Only time will tell whether increasing the area where *E. maimaiga* is established will lead to constant lower populations of the gypsy moth in North America.

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Table 1. Infectivity of *Entomophaga maimaiga* conidia to Lepidoptera species within various suborders and families, in the laboratory, 1992.

Suborder Family	Species Tested	Species Infected	Average percentage of Infection Among Infected Species
Papilionoidea	1	0	0
Pyralidoidea	1	0	0
Bombycoidea	5	2	25.5
Geometroidea	12	1	3.0
Noctuoidea	24	10	38.1
Notodontidae	2	2	6.0
Arctiidae	2	1	7.0
Lymantriidae	4	4	81.8
Noctuidae	16	3	11.7

Pesticide Precautionary Statement

This publication reports the application of an insecticide. It does not contain recommendations for insecticide use, nor does it imply that the uses discussed here have been registered. All uses of insecticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

Caution: Insecticides may be injurious to humans, domestic animals, desirable plants, and fish or other wildlife if they are not handled or applied properly. Use all insecticides selectively and carefully. Follow recommended practices for the disposal of surplus insecticides and insecticide containers.

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